NEUROPROTECTIVE EFFECTS OF L-ARGININE AGAINST DEMENTIA INDUCED MURINE MODEL OF NEUROPATHY PAIN

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Abstract:

Objective: The aim of this study is to perceive new strategy in prevention and treatment of neuropathy with nutraceutical commodity which shows long-term effect on neuropathy symptoms with minimum side effects. The present study deals with the Neuroprotective effect of L-Arginine in dementia induced neuropathy pain.

Methods: Allodynia test were measured for all the groups to confirm the development of dementia induced neuropathic pain. Measurement of allodynia was carried out from day 7th and 15th day. Mice were treated with L Arginine at both low (250mg/kg, p. o.) and high doses (500mg/Kg -p. o.). Allodynia of drug treated group was compared on 15th day with vehicle treated group and behavioral test like Hind paw cold Allodynia method and Tail Cold allodynia method were performed.

Results: By dementia Induced Neuropathic Pain Model, Aluminium chloride treated animals developed qualitative signs indicative of neuropathic pain. This was clearly present starting from 7th day and persisting throughout the study. The aluminium chloride treated group allodynia score on 15th day significantly increased in comparison to the group that had not been treated. In behavioral studies, In hind paw cold allodynia treatment By Aluminium Chloride (50 mg/kg, p.o) Cold observation score for 7th day (3.144±0.14) for 15th day (4.08±0.02) and In tail cold allodynia treatment by aluminium chloride (50 mg/kg, p.o) Cold observation score for 7th day (11.9±0.08) for 15th day(6.03±0.05).

Conclusion: L Arginine was found to be effective against neuropathic pain in lower and also in higher dose. In this regard further involvement in this research may provide a better insight in the development of the newer drug for neuropathic pain. Further increase in the duration of study period may provide better insight for its therapeutic potential.

INTRODUCTION

Neuropathic Pain is the pain caused by damage or disease affecting the nervous system. Neuropathic pain can be associated with abnormal sensations called dysesthesia or pain from normally non-painful stimuli (allodynia) (1). It may have continuous and/or episodic (paroxysmal) components. The latter resemble stabbings or electric shocks. Common qualities can be burning or coldness, "pins and needles" sensations, numbness and itching. (2) Although neuropathic pain is thought to be associated with peripheral nerve problems, such as neuropathy caused by diabetes. (3) or spinal stenosis, injuries to the brain or spinal cord will also lead to chronic neuropathic pain. (4)

Anything which leads to loss of function within the sensory nervous system can cause neuropathic pain. (5) As such, nerve problems from carpel tunnel syndrome or similar conditions can activate neuropathic pain.(6)Trauma causing nerve injury, can lead to neuropathic pain. Other conditions which can predispose patients to developing neuropathic pain include diabetes. (7) vitamin deficiencies, cancer, HIV, multiple sclerosis, shingles and cancer.(8)

L ARGININE THERAPEUTIC EFFECTS

Several disorders have been treated using L- Arginine. It has been demonstrated that L Arginine boosts immune responses and speeds up wound healing. Elderly peoples immune systems and wound healing are specifically stimulated by L arginine. For two weeks , Older patients who received 17 g doses of arginine orally saw a considerable improvement in their positive nitrogen balance. Surprisingly, Arginine dramatically lowered the levels of both low density lipoprotein and total serum cholesterol. At the daily arginine intake of 17g, no side effects were noticed. Elderly patients were able to tolerate an even higher amount of arginine – 30g/day. Additionally, the dietary supplement L Arginine enhances performance and exercise capacity in older male bike racers. Dietary supplement of L Arginine reduces the elevated platelet reactivity in hypercholesterolemic individuals and inhibit atherogenesis. Lastly, after experimental angioplasty in rabbits, a food supplement containing L Arginine lowers restenosis. Neurological problems have also been treated using L Arginine and its derivatives. The frequency and severity of stroke like symptoms are dramatically reduced with L Arginine when administered 30 mins of a stroke. Surprisingly, 1.6g of L Arginine added to the diets of people with senile dementia every day for three months enhanced cognitive function by roughly 40%. Surprisingly, the drug effects did not persist after the treatment (9).

Arginine is a -amino acid that is required for protein biosynthesis. Because of its chemical structure and activity, arginine has a wide range of regulatory functions. Studies shows an extensive review of the potential physiological effects of amino acids on the development of AD. Many vital metabolites (such as NO and urea) are derived from it, and their

cytoprotective and antioxidant properties are now well understood. The cationic properties of arginine's guanidine group contribute to its ability to undergo protonation.

The ability of arginine derivatives and the amino acid itself to regulate peroxidation processes in membranes is well established . Arginine clearly reacts directly with the superoxide anion-radical, which could explain its protective effects under extreme conditions. Furthermore, arginine regulates cell division and hormone release, aids in wound healing and ammonia removal, and has a variety of immune functions. According to epidemiological studies, daily dietary arginine intake is inversely related to Alzheimer's disease morbidity. Men consume more arginine than women, which is consistent with men having a lower prevalence of AD and about two-thirds of those diagnosed with AD. Females and the elderly consume 30% less arginine than 20-40-year-olds.

Furthermore, in some recent studies, a moderate decrease in arginine level was detected in CSF and plasma of AD patients. L-Arginine is transported from the circulating blood into the brain by the Na+-independent cationic amino acid transporter (CAT1) expressed at the BBB in healthy individuals. The L-arginine influx transport at the rat BBB was found to be saturable, with a Michaelis-Menten constant (Km) value of 56 M. The physiological serum concentration of L-arginine in rodents (about 170 M) and humans (about 100 M) is significantly higher. As a result, because L-arginine in mammals is primarily derived from renal de novo synthesis and dietary intake, CAT1 at the BBB is important serves as the sole source of L-arginine to the brain. NO is traditionally synthesised from the amino acid precursor, L-arginine, by the activity of NO producing enzymes (NOS) . NO mimetics have been proven in recent investigations to reduce cognitive impairments and plaque deposition in experimental animals. L-arginine participates in various metabolic pathways in the human body and is the only substrate in the production of NO, which plays important roles in a variety of physiological processes in the central nervous system as well as in diabetes. A recent study discovered that brain arginine metabolism changes with age, which is linked to the pathophysiology and progression of Alzheimer's disease. Given the significance of the dietary metabolic syndrome in Diabetes.

The current study investigates the direct effect of L arginine on pain functions in a murine model of dementia-induced neuropathy pain(10).

MATERIALS USED:

DRUGS AND CHEMICALS

- L-Arginine, Aluminium Chloride, Sodium Chloride, Pregablin are used in the study

L-ARGININE

L-Arginine is an amino acid that is used in the biosynthesis of proteins. (11) It contains an amino group an carboxylic acid group, and a side chain consisting of a 3-carbon aliphatic straight chain ending in a guanidino group.(12)

PREGABLIN

Pregablin is structurally similar to gamma-aminobutyric acid (GABA)-an inhibitory neurotransmitter. (13) Pregablin exerts its effects by binding to the 2 gamma subunit of voltage dependent calcium channels. It is used to treat the neuropathy pain. (14)

PREPARATION OF DRUG SOLUTION: Drug was dissolved in 0.9%saline and administered to animals through p. o. route. (15)

Assessment of effectiveness of L Arginine on Dementia induced neuropathic pain

Dementia induced neuropathic pain was performed following the method of Natarajan et al., 2013. Mice were first treated with Aluminium chloride (50 mg/kg, p.o). Administration of aluminium chloride at a dose of 50mg/kg., p.o causes dementia induced neuropathy. (16) Then we administer L Arginine at both low (250mg/kg, p.o) and high doses (500mg/kg, p.o) for fifteen days. Measurement of allodynia was carried out from day 7th and 15th day. (17) Allodynia test were measured for all the groups to confirm the development of dementia induced neuropathic pain.(18)Allodynia of drug treated group was compared on 15th day with vehicle treated group in order to confirm the effectiveness of drug in dementia induced neuropathic pain.(19)

ANIMAL GROUPS

The mice were divided in to five groups consisting of 6 Per each group.

- Group 1: Vehicle – Saline (10ml/kg i.p.) for 15 days.
- Group 2: Aluminium Chloride (50mg/kg. p.o) for 15 days.
- Group3: Aluminium Chloride(50mg/kg.p.o)+ L-Arginine (250mg/kg.p.o) for 15 days.
- Group4: Aluminium chloride(50mg/kg.p.o)+ L-Arginine (500mg/kg.p.o) for 15 days.
- Group5: Aluminium Chloride (50mg/kg.p.o)+ Pregablin(30mg/kg.p.o)for 15 days

Behavioral Tests

Hind paw cold Allodynia (Choi et al.,1994)

Cold-allodynia was tested by an acetone spray test modified from that described by Choi et al., 1994. With the mice standing upon the perforated floor, 250 µl of acetone was squirted onto the mid-plantar surface of the hind paw. (20) The response was observed and classified according to the following scale.

1. No visible response.
2. Startle response without paw withdrawal.
3. Clear withdrawal of the paw.
4. Prolonged withdrawal (duration 5-30 (sec)) often combines with flinching and licking of the paw.
5. Prolonged repetitive withdrawal (>30(sec)) and /or vocalization.

Tail Cold allodynia (Sharma et al., 2008)

Cold allodynia was assessed using tail immersion test. The tail of the mice was immersed in a water bath at 10℃.

Mice were divided in to groups of six animals each. The lower 5cm portion of the tail was immersed in a beaker of water maintained at 10℃. The time in seconds for tail

withdrawal from the water was taken as the reaction time, with a cut-off time of immersion at10 sec. The reaction time was measured 1 hour before and after oral administration of L-Arginine (250mg/kg. p.o. & 500mg/kg, p.o.) or distilled water (10 ml/kg).
The cut off- latency was found to be 10(sec)

EXPERIMENTAL ANIMALS

Adult male albino mice weighing 25-35g were used in the pharmacological studies. The inbred animals were taken from the animal house in Vels institute of science technology and advanced studies (VISTAS). Pallavaram, Chennai-35. The animals were housed in a group of 6 per cage. They were maintained in well-ventilated room temperature with relative humidity of 45-55% and natural 12h:12hday night cycle in propylene cages. They were fed balanced rodent pellet diet from poultry research station, Nandanam, Chennai-35 and tap water and libitum throughout the experimental period. All the experiments were carried out between 10:00am to 5:00pm.The animals were housed for one week prior to the experiments to acclimatize to laboratory temperature. Food and water were not withdrawn 3hrs before and during the experiment. The experimental protocol was approved by the Institutional Animal Ethical Committee.

RESULTS

Dementia Induced Neuropathic Pain Model

All the aluminium chloride treated animals developed qualitative signs indicative of neuropathic pain. This was clearly present starting from 7th day and persisting throughout the study even when noticed on 15th day. Table 1 showed aluminium chloride treated group allodynia score on 15th day was significantly increased in comparison to 7th day after administering Aluminium Chloride indicating the development of allodynia.

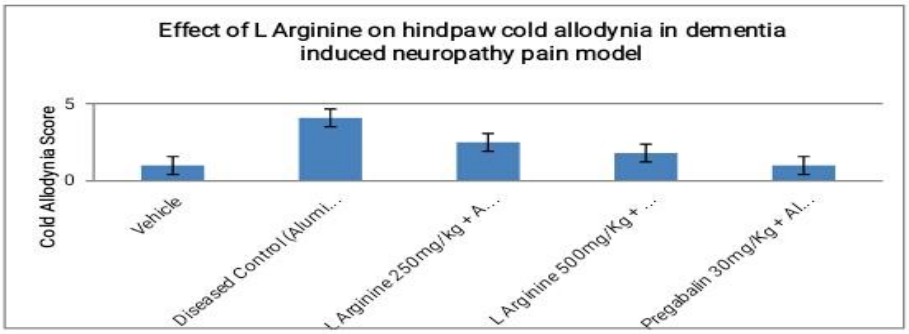
Effect of L Arginine on Cold allodynia on hind paw

A cold stimulus applied to the lateral plantar side of the paw by evaporation of drop of acetone, evoked a substantial increase in duration of flexion withdrawal in the diseased group. The diseased control group developed a marked hypersensitivity to acetone with a significantly increase in the allodynia score. Whereas, L Arginine at both the dose level showed significant decrease in score in comparison to vehicle treatment. Results were also comparable with that of standard (figure 1).

Table 1: Development of Cold allodynia in Aluminium chloride induced dementia model

S. No	Treatment	Cold observation score	
		7th day	**15th day**
1	Aluminium Chloride (50 mg/kg, p.o)	3.144±0.14	4.08±0.02***

Figure 1: Effect of L Arginine on Cold allodynia on hindpaw in Aluminium chloride induced dementia model



Statistical significance test was done by one way ANOVA followed by Dunnets test. Values are mean± S.E.M of 6 animals per group. Comparison was made between vehicle Vs 15th day of all the groups. ***P< 0.001*significant, ** more significant & *** most significant.

Effect of L Arginine on cold allodynia by tail immersion test

Animals showed significant reduction in the tail flick latency on the end of 15 th day as compared to 7th day. L Arginine (250 mg/kg and 500 mg/kg) significantly reversed the cold allodynia in animals (figure 2). Results were comparable with that of the standard.

Table 2: Development of cold allodynia in Aluminium chloride induced dementia Model

S. No	Treatment	Tail flick latency(sec)	
		7^{th} Day	15^{th} Day
1	Aluminium Chloride (50 mg/kg, p.o)	11.9±0.08	6.03±0.05***

Statistical significance test was done by one way ANOVA followed by Dunnet's t test. Values are mean± S.E.M of 6 animals per group. Comparison was made between vehicle Vs drug treatment for 0 day and 15th day of all groups. ***P<0.001 *significant, ** more significant & *** most significant

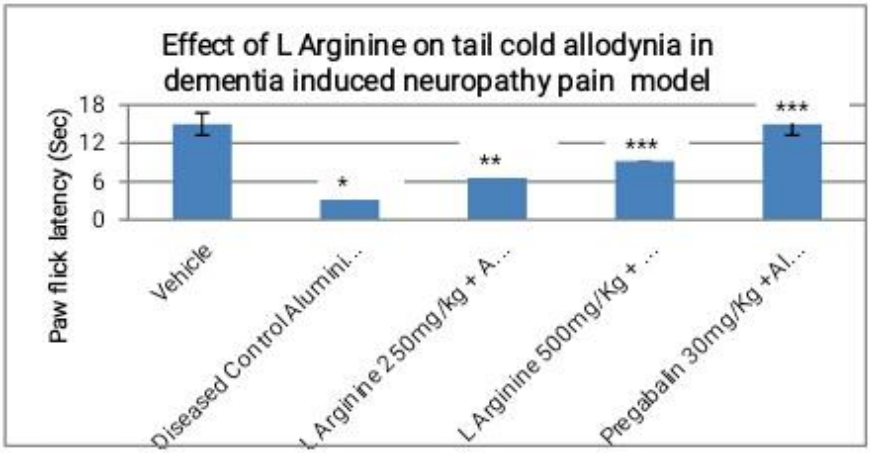


Figure 2: Effect of L Arginine on tail cold allodynia in dementia induced neuropathic pain

DISCUSSION:

Aminoacids are required for neural signalling and energy delivery. The Proper Aminoacid balance is essential for appropriate brain function. As a result, changes in their metabolism may have impact on Neurodegenerative process. In recent studies postmortem brains show a variety of changes in amino acid levels, with a slight drop in arginine levels seen in the CSF and plasma. As a result, we suggest that arginine has a role in the aetiology of the neurodegenerative disease and that it could be utilised to cure it. In comparison to other cells, neurons rely heavily on oxidative phosphorylation for energy and extremely sensitive to oxidative stress. As a general rule, oxidative stress rises with age. The capacity of neurons to maintain redox balance drastically diminishes as age- related neurodegeration progresses, particularly as dementia progresses, leading to mitochondrial malfunction, free radical build up, and neuronal damage. As a result, it was proposed that increasing oxidative damage is the root cause of disease. There is widespread agreement that L Arginine, through its antioxidant properties, can protect neurons from oxidative stress. Furthermore, recent research on a novel rodent model has shown that the development of AD symptoms is associated with a significant reduction in global arginine bioavailability, and that intervening in arginine metabolism via inhibition of ornithine decarboxylase protects animals from AD like pathology and improves cognitive function in mice. L Arginine has been shown to improve resistance to oxidative stress in many animals. The chemical increases the lifetime of C. elegans under oxidative and thermal stress and has free radical scavenging properties. Arginine is a direct precursor of Nitric Oxide (NO). NO has been proven in many cell cultures to act as an antioxidant, protecting cells from ROS induced damage. It has been proposed that the mechanism for NO protection is the interception of ROS and metallo-oxo compounds created by NO.

The current study major goal was to investigate the behavioral study involving the L Arginine in the disease development. Earlier studies shows that the cognitive benefit of L Arginine administration is associated with the cytoprotective and antiapoptotic potentials of arginine

rather than the reduction of amyloid plague formation or activation of neuroplasticity for long term potentiation.

Present findings support the hypothesis that L Arginine protects neurons against dementia induced neuropathy pain and decreases apoptosis intriguing therapeutic function of L Arginine as a strong metabolic agent interfering with the redox system and lowering apoptosis in the development of dementia induced neuropathy pain.

We think that our study will contribute in the rational creation of therapeutic medicines for use in the treatment of a variety of pertinent human ailments.

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Declaration of conflict of interest(s)

"There are no potential conflicts of interest to declare."

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